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Favorable morphological and cultural conditions for mycelial growth of *Sclerotium rolfsii*(curzi) C.C Tu & Kimber, causing stem blight of tomato

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Abstract
Tomato is subjected to a number of diseases at all the stages of its development from nursery to the consumption of the tomatoes. Among all the diseases infecting tomato, the stem blight of tomato is the most severe threat for the tomato industry and also for foreign exchange earnings. This disease is incited by number of pathogens but the major causal organism is *Sclerotium rolfsii* (curzi) C.C Tu &Kimber. Out of five solid media were used potato dextrose agar (PDA) was the best solid media for the growth of mycelium and highest sclerotial production was observed in sabouraud's dextrose agar(SDA). Starch and dextrose were recorded best carbon source while sodium nitrate and potassium nitrate were found to be the best nitrogen source for the growth of *Sclerotium rolfsii*. The pathogen grew between 5ºC to 40ºC, with maximum mycelial growth at 30ºC, with highest sclerotia formation. The hydrogen ion concentration 6.5 showed maximum mean diametric growth and sclerotial production.

Keywords: *Sclerotium rolfsii*, growing media, carbon and nitrogen sources, sclerotial bodies, temperature and pH levels

Introduction
Tomato (*Lycopersiconesculentum L.*) is an important and most widely consumed vegetable which is rich in nutrients and cultivated in nearly every country in the world. (Sahana *et al.* (2017) [15]. due to its role as an essential ingredient in a wide variety of fresh, cooked or processed foods. It belongs to the Solanaceae family, which includes several other commercially significant plants. Tomato is cultivated in nearly every country in the world, China, India, USA, Turkey, Spain and Brazil are the leading tomato producing countries. Worldwide tomato production amounts to 182.30 million metrictons from an area of 4.84 million ha (FAO, 2017) [5]. Tomato is known to be the world's third largest vegetable crop after potatoes and onions. India is the second largest producer of tomatoes in the world after China. According to the department of agriculture, cooperation and farmer welfare of India, tomatoes cover an area of 814 thousand hectares with annual production of20.5 million metric tons in 2018-2019**.**The annual production of tomato India is 20515MT/814 Ha (NHB 2018-19).
Commercially cultivated tomato fruit may vary in colour, size and shape (Vaughan and Geissler, 1997). The fruit contains a large amount of water, vitamins and minerals, a low amount of protein, fat and some carbohydrate. It also includes carotenes such as lycopene (which gives the fruit its predominant red color) and beta-carotene (which gives the fruit its orange color). The disease caused by *Sclerotium rolfsii*, soil borne fungi that causes foot rot or collar rot in tomatoes, has become more serious among plant pathogenic fungi. It is known to be pathogenic to nearly 500 species of plants. The annual loss of vegetables due to the pathogen is 10%, the largest loss is due to the fungal pathogen *S.rolfsii* is a polyphagous and most harmful fungus bearing soil, (Sahana *et al*, 2017) [15] and was first reported to Rolfs (1892) as a cause of tomato blight in Florida, (Bhuiyan *et al.* 2012. and Nagaraja *et al*. 2012) [3, 10].

Materials and methods

Physiological studies

Studies on different solid media for viability and maintenance of *Sclerotium rolfsii*

Five different solid media viz. Potato Dextrose Agar, Nutrient Agar, Cornmeal Agar, Sabouraud's Dextrose Agar and Czapek's Dox Agar were tested. The best medium was used for further maintenance, multiplication and selection of suitable medium for physiological studies.

Different solid media

Different solid media and their composition usedduring the course of present investigation are given below.

Solid media.

a) Potato Dextrose Agar (PDA) Medium

Peeled potato slices : 200 g
Dextrose : 20 g
Agar : 20 g
Distilled water : 1000 ml
pH : (6.0)

b) Corn meal agar (CMA) medium

Corn meal, infusion from : 50 gm
Agar : 15 gm
Distilled water : 1000 ml
pH : (6.0)

c) Sabouraud dextrose agar (SDA) medium

Mixture of peptic digest of animal tissues
& pancreatic digest of casein (1:1) : 10 gm
Dextrose : 40 gm
Agar : 15 gm
Distilled water : 1000 ml
pH : 5.6 ± 0.2

d) Nutrient agar medium (NA)

Peptic digest of Animal tissues : 5 gm
Sodium chloride : 5 gm
Beef extract : 1.5 gm
Yeast extract : 1.5 gm
Agar : 15 gm
Distilled water : 1000 ml
pH : 7.4 ± 0.2
temperature : 25ºc

e) Czapek dox agar medium (CDA)

Sucrose : 30 gm
Sodium nitrate : 3 gm
Potassium phosphate (dibasic) : 1 gm
Magnesium sulphate : 0.5gm
Potassium chloride : 0.5gm
Ferrous sulphate : 0.01 gm
pH : 7.3 ± 0.2
temperature : 25ºc

Measure of growth

For determining the variation in the colony growth of *Sclerotium rolfsii*, the colony growth of fungus in each petri plate was measured when entire control petri plate was covered by fungus. The colony growth was measured along two diameters at right angles and averaged.

Study of colony and sclerotial characters

Observation for various cultural and morphological characteristics of mycelia and sclerotial formation viz., mycelia colour, margins, growth pattern, distribution of mycelia growth, days to sclerotial initiation(days), days to completion of sclerotial formation(days),distribution of sclerotia over media, shape, colour and texture, diameter(mm), test weight of 100 sclerotial bodies in mg, no.offruting bodies per plate. Were recorded 55days after incubation. The mature sclerotial bodies in each patri plate were harvested separately using fine camel brush and counted. The sclerotial size of *Sclerotium rolfsii* was measured by micrometry method. The microscope was calibrated with stage and ocular micrometer at a magnification of 100 X (eye piece:10X:objective: 10X). The size of 25 sclerotial bodies were measured in each replication and averaged.

Influence of different carbon sources on growth of mycelium and sclerotialformation

To study the effect of different carbon sources on the mycelia growth of test fungus, the carbon source present in the solid basal medium was substituted by different carbon sources viz. (Dextrose, Fructose, Sucrose, starch and Control). Glucose – asparagine agar media was used as the basal media. The carbon source in the basal medium was replaced by different carbon compounds inorder to provide or supply an equal amount of carbon per liter as obtained from the carbon source originally standardized for a particular medium. The quantity of each carbon source per liter of the medium is shown in below fallowing table. All the treatments were replicated five times and sterilized at 15 psi pressure for 15 minutes and inoculated with 6 mm mycelia bits of the test fungus after cooling. These were further incubated at 25 ± 2°C in BOD incubator.

Amount of different carbon sources added to the basal medium individually in order to replace the carbon source of the media

Carbon source	Molecular weight	Quantity (g*) per litre
Glucose –asparagine agar media		
Starch($C_6H_{12}O_6$)	182.17	30.33
Glucose($C_6H_{12}O_6$)	180.15	29.99
Sucrose($C_{12}H_{22}O_{11}$)	342.30	28.50
Fructose ($C_6H_{12}O_6$)	180.16	45.50
Dextrose ($C_6H_{14}O_7$)	198.17	50.00
* To replace 30.33 g Starch in Glucose –asparagine agar media		
* To replace 30g Glucose in Glucose –asparagine agar media		
* To replace 28.5 g Sucrose in Glucose –asparagine agar media		
* To replace 45.50 g Fructose in Glucose –asparagine agar media		
* To replace 50.00 g Dextrose in Glucose –asparagine agar media		

Observations: Radial growth rate (mm/day) were recorded along with the type of growth (fluffy, dense, strand/normal) in various treatments and data analyzed statistically.

Influence of different nitrogen sources on growth of mycelium and sclerotial formation

To study the effect of different nitrogen sources on the mycelial growth of test fungus, the nitrogen source present in the solid basal medium was substituted by different nitrogen sources viz. (Sodium nitrate, Potassium nitrate, Ammonium chloride, Urea and Control). Glucose –asparagine agar media was used as the basal media. The nitrogen source in the basal medium was replaced by different nitrogen compounds so as to provide or supply an equal amount of nitrogen per liter as obtained from the nitrogen source originally standardized for a particular medium. The quantity of each nitrogen source per liter of the medium is shown in below fallowing table. All the

treatments were replicated five times and sterilized at 15 psi pressure for 15 minutes and inoculated with 6 mm mycelia bits of the test fungus after cooling. These were further incubated at 25 ± 2°C in BOD incubator.

Amount of different nitrogen sources added to the basal medium individually in order to replace the nitrogen sources of the media

Carbon source	Molecular weight	Quantity (g*) per litre
Glucose-asparagine agar media		
Ammonium Chloride (NH_4C_l)	53.49	0.73
Sodium nitrate ($NaNO_3$)	83.99	1.39
Potassium nitrate (KNO_3)	101.10	1.37
Urea (CH_4N_2O)	60.06	8.6
Asparagines ($C_4H_8N_2O_3$)	132.12	0.5
* To replace 8.6 g Urea in Glucose –asparagine agar media		
* To replace 0.73 g Ammonium Chloride in Glucose –asparagine agar media		
* To replace 0.5 g Asparagines in Glucose –asparagine agar media		
* To replace 1.39 g Sodium nitrate in Glucose –asparagine agar media		
* To replace 1.37 g Potassium nitrate in Glucose –asparagine agar media		

Observations: Radial growth rate (mm/day) were recorded along with the type of growth (fluffy, dense, strand/normal) in various treatments and data analyzed statistically.

Study of Colony and sclerotial characters on both carbon and nitrogen sources.

Observation for various cultural and morphological characteristics of mycelia and sclerotial formation viz., mycelia colour, margins, growth pattern, distribution of mycelia growth, days required for the initiation of sclerotial bodies(days), days to complete sclerotial formation (days),distribution of sclerotial over media, shape, colour and texture, diameter(mm), test weight of 100 sclerotia bodies in mg, no. offruting bodies per plate were recorded 55days after incubation. The mature sclerotial bodies in each petri plate were harvested separately using fine camel brush and counted. The sclerotial size of *Sclerotium rolfsii* was measured by micrometry method. The microscope was calibrated with stage and ocular micrometer at a magnification of 100 X (eye piece: 10X:objective: 10X). The size of 25 sclerotial bodies were measured in each replication and averaged.

Effect of different temperature requirements on mycelia growth of *Sclerotium rolfsii*

To study the effect of different temperature regimes, petri plates containing potato dextrose agar (PDA) medium along with inoculum of test culture were incubated at different temperatures viz. (5℃,10℃,15℃, 20℃, 25℃, 30℃,35^{0}C and 40℃) in different incubators for 6 days. Each treatment was replicated four times and data were analyzed statistically.

Effect of different pH levels on the mycelial growth of *Sclerotium rolfsii*

In this experiment, the basal medium(PDA) was adjusted at different pH levels viz., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 with the help of a microprocessor-based pH meter using N/10 HCl or N/10 NaOH and finally inoculated with 6 mm mycelial bits of the culture and incubated at 25 ± 2°C in BOD incubator for 6 days in BOD incubator. Each treatment was replicated four times. Types of growth were also recorded and data obtained were subjected to statistical analysis.

Results and discussion

Physiological studies

Solid media

The pathogen was grown on five different solid media which were selected for this analysis. Observations on the radial growth rate of *S.rolfsii* in four replications were reported at the end of the 5th day of inoculation. Irrespective of the media used potato dextrose agar (PDA) was found to be the most suitable culture medium for the growth of *Sclerotium rolfsii.* Statistical analysis of the data showed that the radial growth rate of potato dextrose agar (PDA) was found to be statistically higher than that of other cultural media and potato dextrose agar (PDA), saubouard dextrose agar(SDA) is statistically the same as the growth rate. Whereas nutrientagar (NA) medium was found to be a non-preferable culture medium with a minimum radial growth rate.

Intervals of 24, 48, 72, 96 and 120 hrs PDA was found to be the most suitable culture medium for the growth of *Sclerotium rolfsii*, with a maximum radial growth rate. Similarly, in all intervals SDA was found to be statistically equal to the growth rate of potato dextrose agar (PDA). Where nutrient agar (NA) has been found to be a non-preferable culture medium for the growth of *Sclerotium rolfsii*.

Effect of different solid media on the growth of mycelium (mm/day) of *Sclerotium rolfsii*

Solid media	24(hrs)	48(hrs)	72(hrs)	96(hrs)	120(hrs)
PDA	13.25	37.50	68.50	89.75	90.00
CMA	12.75	32.75	52.50	71.50	89.50
SDA	10.75	32.50	57.50	86.00	89.75
NA	8.50	12.75	29.50	40.50	46.25
CDA	13.00	36.50	65.50	87.75	89.50
C.D.	1.43	1.78	1.80	1.82	1.08
SE(m)	0.47	0.59	0.59	0.60	0.35

Effect of different solid media on the growth of mycelia and sclerotialbodies formation.

Colony and sclerotial characters	PDA	CMA	SDA	NA	CZA
Mycelial colour	Pure white	Dull white	Pure white	Dull white	Pure white
Mycelia margins	Smooth	Smooth	Filamentous	Smooth	Smooth
Growth pattern	Compact	Compact	Fluffy	Cloudy	Compact
Distribution of mycelia growth	Thick	Thin	Thick	Irregular	Thick
Days to Sclerotial initiation (days)	9	20	9	16	14
Days to completion of sclerotial formation (days)	40	55	35	52	50
Distribution over media	All over plate	All over plate	Periphery	Periphery	All over plate
Shape of Scleriotial bodies	Round	Round	Irregular	Irregular	Round
Fruiting body colour and texture	Brown	Light brown	Dark brown	Light brown	Dark brown
Fruiting diameter (mm)	1.40	1.33	1.36	1.00	1.13
Sclerotial bodies weight(100 no.) in mg	217.33	120	254.66	67.66	188.66
No.of fruiting bodies per plate	227.33	31.00	243.33	15.33	145.66

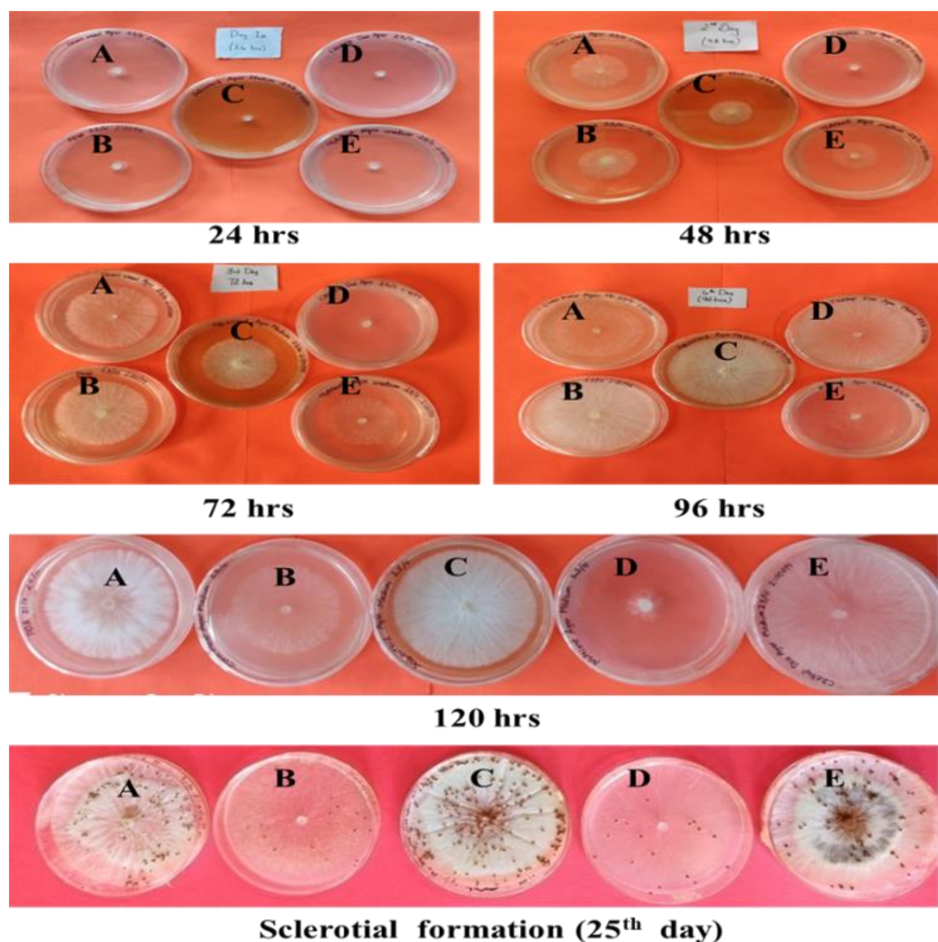

Plate 2: Effects of different solid media on the growth of mycelium and sclerotial bodies formation. (A) PDA, (B) CMA, (C) SDA, (D) NA and (E) CZA.

During present studies, five different solid media were tested for culturing of *Sclerotium rolfsii*.Out of which, potato dextrose agar(PDA) was observed maximum mycelia growth followed by saubouard dextrose agar(SDA) which was conformation with the work done by Hyeuk and Seuk (2002) [9], Sahana *et al.* (2017) [16] and Kushwah *et al.* (2019) who tested different solid media for culturing of *Sclerotium rolfsii* and concluded that potato dextrose agar(PDA) medium and saubouard dextrose agar(SDA)supported the best mycelia growth among the solid media. potato dextrose agar (PDA) was found to be the best supporting medium for *S. rolfsii* by Naidu (2000) [7]; Amarsingh and Singh, (1994); Gupta and Sharma, (2004); Gaur *et al.* (2005) [6] and Raoof *et al.* (2006) [14].

Influence of different carbon sources on growth pattern and sclerotial bodies formation

The selected isolate of *Sclerotiumrolfsii* was grown on five different carbon s ources selected for the present study. The observations on the radial growth rate of *Sclerotium rolfsii* was recorded after completion of the 5th day of inoculation. Irrespective of carbon sources used**,** starch was found to be the most suitable carbon source for raising the cultures of *Sclerotium rolfsii*. Statistical analysis of data revealed that the radial growth rate on starch was found to be statistically superior than the other carbon sources where as dextrose and fructose were statistically at par with each other. Sucrose was found to be the non-preferable carbon source with a minimum radial growth rate.

For *Sclerotium rolfsii*, Starch was found to be most suitable carbon source. The radial growth rate on dextrose was found to be statistically at par with growth rate on fructose. sucrose

was found to be the non preferable carbon source with a minimum radial growth rate.

Effect of different carbon sourceson mycelia growth (mm/day) of *Sclerotium rolfsii*

Carbon sources	24(hrs)	48(hrs)	72(hrs)	96(hrs)	120(hrs)
Fructose	13.50	35.25	56.75	85.25	86.50
Dextrose	13.50	33.50	55.00	84.75	87.00
Starch	16.25	40.00	57.25	80.25	87.25
Sucrose	9.75	20.00	46.00	63.75	82.50
Control	12.75	38.25	55.75	83.25	82.75
C.D.	2.49	2.31	1.77	1.75	2.13
SE(m)	0.82	0.76	0.58	0.57	0.70

Influence of different carbon sources on growth pattern and sclerotial bodies formation

Colony and sclerotial characters	Fructose	Dextrose	Starch	Sucrose	Control
Mycelial colour	Pure white	Dull white	Dull white	Pure white	Pure white
Mycelia margins	Filamentous	Smooth	Smooth	Filamentous	Filamentous
Growth pattern	Thick strand	Thin strand	Thin strand	Compact	Thin strand
Distribution of mycelia growth	Thick strand	Thin strand	Thin strand	Compact	Thin strand
Days to Sclerotial initiation (days)	11	11	10	10	10
Days to completion of sclerotial formation (days)	45	45	40	50	45
Distribution over media	Center and margins	Center and margins	Center and margins	Margins	Center and margins
Shape of Scleriotial bodies	Round and irregular	Oval and round	Round and irregular	Oval to round	Round and spherical
Fruiting body colour and texture	Dark brown	Dark brown	Light brown	Light brown	Dark brown
Fruiting diameter (mm)	1.40	1.33	1.36	1.00	1.13
Sclerotial bodies weight(100 no.) in mg	45.33	47	46	47.66	45
No.of fruiting bodies per plate	25.33	13.33	21	7.333	20

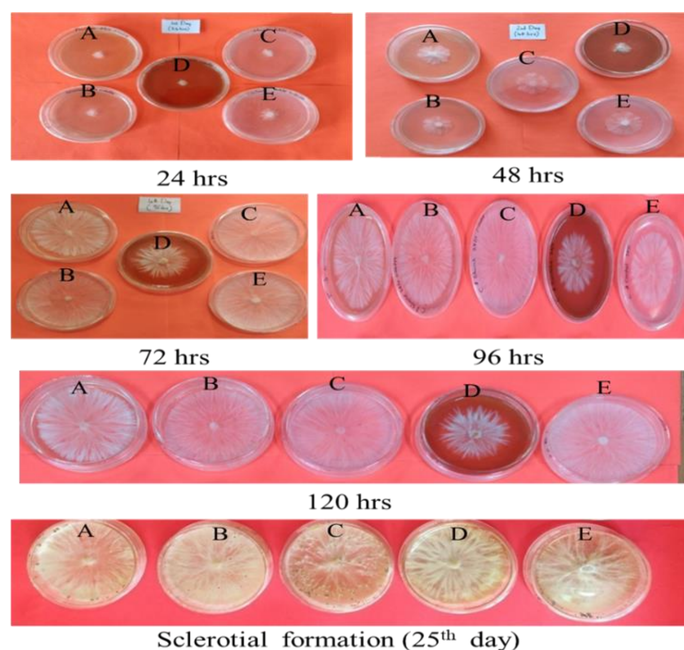

Plate 3: Influence of different carbon sources on growth of mycelium and sclerotial formations (A): Fructose, (B): dextrose (C): Starch, (D): Sucrose and (E): Control

Carbon is very important factor that control the growth of fungi. In the present study, Starch was found to be the best carbon source followed by dextrose for culturing of *Sclerotium rolfsii*. Present findings regarding carbon source are in accordance with observations of Hussain *et al*. (2003) and Bhagat (2011) who reported starch gave the highest yield followed by dextrose.

Influence of different nitrogen sources on mycelia growth of *Sclerotium rolfsii*.

The selected isolate of *Sclerotium rolfsii* was grown on five different nitrogen sources selected for the present study. The observations on the radial growth rate of *Sclerotium rolfsii* was recorded after completion of the 5th day of inoculation. Irrespective among all the nitrogen sources used, sodium nitrate was found to be the most suitable nitrogen source for raising the cultures of *Sclerotium rolfsii*. Statistical analysis of data revealed that the radial growth rate on sodium nitrate was found to be statistically superior to other nitrogen sources. pottasium nitrate and ammonium chloride was statistically at par with each other. Whereas, urea was found to be the non preferable nitrogen source with a minimum radial growth rate.

Influence of different nitrogen sources on mycelial growth (mm/day) of *Sclerotium rolfsii*

Nitrogen sources	24(hrs)	48(hrs)	72(hrs)	96(hrs)	120(hrs)
Ammonium chloride	17.25	34.50	71.50	76.50	86.00
Potassium nitrate	22.00	41.50	75.00	86.25	88.50
Sodium nitrate	25.00	45.75	76.25	88.00	90.00
Urea	0.00	0.00	0.00	0.00	0.00
Control	12.75	31.00	46.50	67.00	86.75
C.D.	2.47	2.18	2.03	1.83	1.09
SE(m)	0.81	0.72	0.67	0.60	0.36

Influence of different nitrogen sources on growth of mycelium andsclerotia formation

Colony and sclerotial characters	Ammonium chloride	Potassium nitrate	Sodium nitrate	Urea	Control
Mycelial colour	Pure white	Dull white	Pure white	_	Pure white
Mycelia margins	Smooth	Smooth	Smooth	_	Smooth
Growth pattern	Thick strand	Thin strand	Fluffy	_	Thick strand
Distribution of mycelia growth	Thick strand	Thin strand	Thin strand	_	Thick strand
Days to Sclerotial initiation (days)	15	12	10	_	11
Days to completion of sclerotial formation (days)	45	45	40	_	45
Distribution over media	Only at center	Center and margins	Only at center	_	Center and margins
Shape of Scleriotial bodies	Round and irregular	Minute spherical	Minute spherical	_	Round and spherical
Fruiting body colour and texture	Dark brown	Dark brown	Light brown	_	Dark brown
Fruiting diameter (mm)	1.40	1.33	1.36	_	1.13
Sclerotial bodies weight(100 no.) in mg	45.33	47	46	_	45
No.of fruiting bodies per plate	11.66	8	11	_	26.66

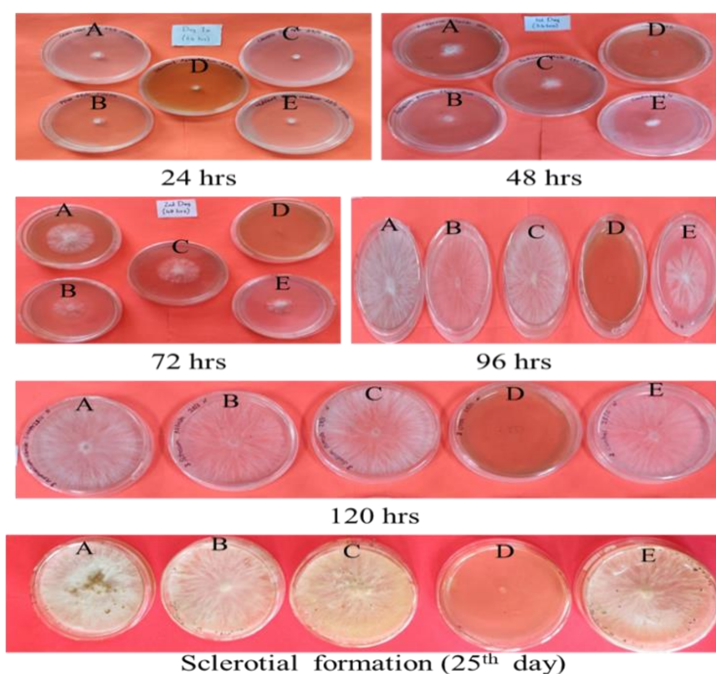

Plate 4: Influence of different nitrogen sources on growth of mycelium and sclerotial formations (A): Ammonium chloride, (B): Pottasium nitrate (C): Sodium nitrate, (D): Urea and (E): Control

In the present study, sodium nitrate was found to be the best nitrogen source followed by potassium nitrate for culturing of *Sclerotium rolfsii.* Present investigation regarding nitrogen sources are in accordance with observations of Hussain *et al.* (2003). Similar results were obtained by Hussain *et al.* (2003); Venkatesh and Muthukumar (2013) [11] who reported maximum growth was observed in sodium nitrate followed by potassium nitrate.

Effect of different temperature regimes on mycelial growth of*Sclerotium rolfsii*

The selected isolate of *Sclerotium rolfsii* was grown on eight different temperature ranges selected for the present study. The observations on the radial growth rate of *Sclerotium rolfsii* was observed at the end of the 5th day of inoculation. Irrespective among all the temperatures,30ºC was found to be the most appropriate temperature for growing *Sclerotium rolfsii* cultures. Statistical analysis of the data showed that the radial growth rate at 30ºC was statistically higher than the other temperature ranges at 25ºC. The minimum radial growth rate was 10ºC. No radial growth of mycelium was developed at 5ºC and 40ºC.

Effect of different temperature levels on mycelial growth (mm/day) of *Sclerotiumrolfsii*

Temperature (ºC)	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs
5	6.00	6.00	6.00	6.00	6.00
10	6.00	6.00	7.25	9.75	14.00
15	6.00	6.50	12.50	18.75	25.25
20	6.00	9.50	20.00	31.00	42.50
25	6.50	16.75	29.25	43.25	65.50
30	10.25	29.75	47.25	65.00	90.00
35	6.00	6.50	12.50	18.75	25.00
40	6.00	6.00	6.00	6.00	6.00
C.D.	0.40	0.87	1.28	1.08	1.07
SE(m)	0.14	0.30	0.44	0.37	0.36

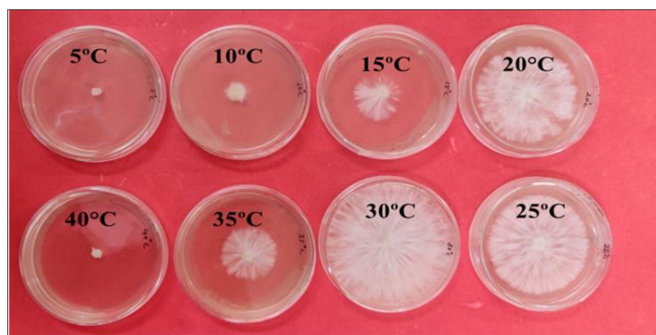

Plate 5(a): Effect of different temperature requirements on mycelial growth of *Sclerotiumrolfsii*

Temperature is a very important environmental factor regulati ng the growth of the fungi. In this study, *Sclerotium rolfsii* were found to grow best in temperature ranges 25ºC to 30^{0}C under *in-vitro* conditions. The present finding regarding optimum temperature level are in accordance with findings of Fakher Ayed *et al.* (2018) who also reported similar results. Similarly, A temperature of 25-30˚C has also been found suitable for the growth of *Sclerotium rolfsii*, Muthu kumar *et al.* (2019) [12]. The wide range of variation in temperature requirement can be attributed to the ecological diversity of *Sclerotium rolfsii*. Shiva Kant Kushwah *et al.* (2019) reported temperature of 30^0 C as the best temperature for *Sclerotium rolfsii.*. The optimum temperature at 30^{0}C has been reported to give maximum mycelia growth by Jin-Hyeuk and Chang-Seuk (2002) [9].

Mycelial growth of *Sclerotiumrolfsii* at various hydrogen –ion concentrations

The isolated *Sclerotium rolfsii* was grown on seven different hydrogen –ion concentration is selected for the present study. The observations on average growth (mm) rate of *Sclerotium rolfsii* was recorded after completion of 5days after incubation. Irrespective among all the hydrogen –ion concentrationsused, pH 6.5 was found to be the most suitable for the growth of *Sclerotium rolfsii*. Statistical analysis of data revealed that the average growth (mm) on pH 6.0 were found to be statistically at par with pH 6.5. Whereas, the minimum average growth was observed at pH 8.5.

Effect of different pH levels on the mycelial growth (mm/day) of *Sclerotium rolfsii*

pH	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
5.5	15.50	34.50	41.50	51.75	64.25
6.0	23.50	43.00	58.00	71.25	84.75
6.5	29.75	49.25	65.00	79.00	90.00
7.0	23.50	43.75	52.25	68.00	77.25
7.5	17.00	29.75	43.25	51.75	65.25
8.0	7.25	12.00	18.50	21.00	26.00
8.5	6.00	9.50	14.25	19.00	23.50
C.D.	1.46	1.38	1.38	1.30	1.21
SE(m)	0.49	0.47	0.47	0.44	0.41

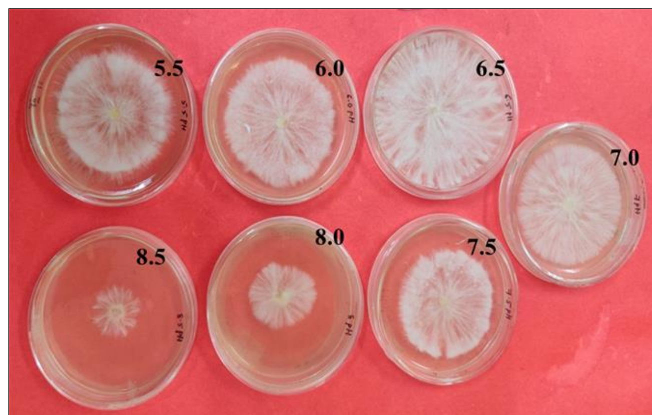

Plate 5(b): Effect of different pH levels on mycelial growth of *Sclerotiumrolfsii*

In the present investigation, *Sclerotiumrolfsii*was found to grow best in acidic environments (pH 5.5 - 6.5). Our findings are in accordance with Hussain *et al* (2003) and Kushwah *et al* (2019). Bhagat (2011); Venkatesh and Muthukumar (2013) [11] reported acidic pH (5.0-6.0) was the best for the growing of *Sclerotium rolfsii.*

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References

1. Ansari MM, Agnihotri SK. Morphological, physiological and pathological variations among *Sclerotium rolfsii* isolates of soybean. Indian Phytopathology. 2000; 53:65-67.
2. Azhar HS, Muhammad INA, Abdul MH. Physiological study of *Sclerotium rolfsii* Sacc, Plant Pathology Journal. 2003; 2:102-106.
3. Bhuiyan MAHB, Rahman MT, Bhuiyan KA. *In vitro* screening of fungicides and antagonists against *Sclerotium rolfsii,* African Journal of Biotechnology. 2012; 11:14822-14827.
4. Fakher A, Hayfa JK, Rania ABA, Mejda DR. Effect of temperatures and culture media on *Sclerotium rolfsii* mycelial growth, sclerotial formation and germination, Journal of Plant Pathology & Microbiology, 2018; 9:8.
5. FAO. 2017. http://www.fao.org/faostat/en/#data/QC(3:00 PM, 12 December 2019).
6. Gaur RB, Sharma RN, Sharma RR, Vinod Singh Gautam. Efficacy of *Trichoderma for Rhizoctonia* root rot control in chickpea. Journal of Mycology and Plant Pathology. 2005; 35:144-150.

7. Harinath Naidu. Crossandra a new host record for *Sclerotium rolfsii*. Indian Phytopathology. 2000; 53:496-497.
8. Indramani Bhagat. Factors influencing mycelial growth of *Sclerotium rolfsii.,* Nepalese Journal of Biosciences. 2011; 1:26-31.
9. Jin-Hyeuk Kwon, Chang-Seuk Park, Stem rot of tomato caused by *Sclerotium rolfsii*in Korea, The Korean Society of Mycology. 2002; 30:244-246.
10. Manu TG, Nagaraja A, Chetan S, Janawad, Hosamani V. Efficacy of fungicides and biocontrol agents against *Sclerotium rolfsii*causing foot rot disease of finger millet, under *In vitro* conditions. Global Journal of Biology Agriculture and Health Sciences. 2012; 1:46-50.
11. Muthukumar A, Venkatesh A. Physiological studies of *Sclerotium rolfsii* Sacc. causing collar rot of peppermint. African Journal of Biotechnology. 2013; 12:6837-6842.
12. Muthukumar A, Udhayakumar R, Naveenkumar R. Effect of nutritional sources on the growth of *Sclerotium rolfsii* causing stem rot of cluster bean., Plant Archives. 2019; 1:1297-1300.
13. NHB. 2019. http://www.nhb.gov.in/statistics (1:00 PM, 12December2019).
14. Raoof MA, Rama Bhadra Raju, Mehtab Yasmeen. Biocontrol potential and shelf life of *Trichoderma viride* for the management of castor wilt. Indian Journal of Plant Protection. 2006; 34:75-80.
15. Sahana NB, Sanath Kumar VB, Thejesha AG. Morphological and cultural studies of *Sclerotium rolfsii* Sacc. causing foot rot disease of tomato, International Journal of Current Microbiology and Applied Sciences. 2017; 3:1146-1153.
16. Sahana NB, Sanath Kumar VB, Thejesha AJ, Kedarnath. *In Vitro* evaluation of botanicals against foot rot (*Sclerotium rolfsii* Sacc.) of tomato. Intrenational Journal of Advanced Biological Research. 2017; 7:214-216.
17. Shiva KK, Sanjeev K, Balkishan C, Ravit S. Effect of different media, ph and temperature on growth and sclerotia formation of *Sclerotium rolfsii* Sacc. causing collar rot of lentil., Chemical Science Review and Letters. 2019; 8:01-05.